REMARKS

The Office Action of January 8, 2008, including the Examiner comments and suggestions, have been carefully considered. Claims 1-7 and 9-12 have been withdrawn. Claim 8 has been amended to correct language deficiencies. Formulas (1), (2), (3) and (4) have been directly incorporated in Claim 8. Dependent Claims 13 and 14 have been added.

Regarding the rejection of Claim 8 under 35 U.S.C. § 102(b) over Kelly et al., this claim has been amended to require administering at least one substance selected from the group consisting of a dokudami extract, a licorice abstract, a compound represented by the formula (1), a compound represented by the formula (2), a compound represented by the formula (3) and a compound represented by the formula (4). The Kelly et al. reference does not disclose a licorice abstract, a dokudami extract or any other compounds in the group of the additives consisting those listed in Claim 8. Accordingly, Claim 8 is not anticipated by Kelly et al.

Kelly et al., additionally, does not suggest the use of a licorice extract in the method(s) of Claims 8, 13 or 14. Accordingly, those claimed methods would not have been obvious in view of Kelly et al.

Regarding the scope of dependent Claims 13 and 14, the independent Claim 1 method stimulates interleukin-8 product production in a mammal for the purpose of treating an infection and/or stimulating immunity. The specification, on page 14, lines 5-7 (et. seq.), discloses the claimed invention there in broad terms. It is known that the production of interleukin-8 (IL-8) is closely related to immunity and inflammatory response, as discussed in the Background Art section in the specification. Furthermore, there are published reports which show the relationship of promoting IL-8 production to treating infection disease. For example, see J.Y. Djue et al., THE JOURNAL OF IMMUNOLOGY, 144, 2205-2210, 1990, which shows IL-8 can act as a stimulator of biologic function in PMN, with affects on infection or inflammation. Also, see

U.S. Patent No. 5,900,235, and Japan published application JP 2001-257942, which show IL-8 is useful in the treatment of infection. These publications have been submitted in an IDS filed April 6, 2008, and a copy of the IDS is enclosed.

Respectfully submitted,

/Richard G. Lione/
Richard G. Lione
Registration No. 19,795
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Enclosure

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CERTIFICATE OF EFS FILING UNDER 37 CFR §1.8

I hereby certify that this correspondence is being electronically transmitted to the United States Patent and Trademark Office, Commissioner for Patents, via the EFS pursuant to 37 CFR §1.8 on the below date:

Date: May 6, 2008 Name: Richard G. Lione, Reg. No. 19,795 Signature: /Richard G. Lione/

BRINKS HOFER GILSON &LIONE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Appln. of:	Hideo Satsu et al.	
Appln. No.:	10/579,323	Examiner: Michael V. Meller
Filed:	May 16, 2006	Art Unit: 1655
For:	IL-8 PRODUCTION PROMOTERS AND USE THEREOF	Conf. No.: 6569
Docket No:	5404/152	

TRANSMITTAL

Mail Stop Amendment
Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

Sir:

Attached is/are:

☒ Transmittal; Second Supplemental Information Disclosure Statement; Form PTO-1449; and Copies of References C2-C3.

Fee calculation:

☐ No additional fee is required.

☐ Small Entity.

☐ An extension fee in an amount of $_____ for a _____-month extension of time under 37 CFR § 1.136(a).

☒ A petition or processing fee in an amount of $180 under 37 CFR § 1.17(p).

☐ An additional filing fee has been calculated as shown below:

	Claims Remaining After Amendment		Highest No. Previously Paid For	Present Extra	Small Entity Rate	Small Entity Add'l Fee	OR	Not a Small Entity Rate	Not a Small Entity Add'l Fee
Total		Minus			x $25=			x $50=	
Indep.		Minus			x 105=			x $210=	
First Presentation of Multiple Dep. Claim					+$185=			+ $370=	
					Total	$		Total	$

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Respectfully submitted,

May 6, 2008
Date

/Richard G. Lione/
Richard G. Lione (Reg. No. 19,795)

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Signature
May 6, 2008
Date of Signature

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Appln. of:	Hideo Satsu et al.		
Appln. No.:	10/579,323	Examiner:	Michael V. Meller
Filed:	May 16, 2006	Art Unit:	1655
For:	IL-8 PRODUCTION PROMOTERS AND USE THEREOF	Confirmation No.	6569
Attorney Docket No:	5404/152		

SECOND SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

In accordance with the duty of disclosure under 37 C.F.R. §1.56 and §§1.97-1.98, and more particularly in accordance with 37 C.F.R. §1.97(c), Applicant hereby cites the following reference(s):

U.S. PATENT DOCUMENTS		
DOCUMENT NUMBER	DATE	NAME
5,900,235	05/04/1999	Gosselin et al.

FOREIGN PATENT DOCUMENTS		
DOCUMENT NUMBER Number-Kind Code (if known)	DATE	COUNTRY
JP 2003-063991	03/05/2003	Japan

OTHER ART – NON PATENT LITERATURE DOCUMENTS
Djeu, Julie Y., "Functional Activation of Human Neutrophils by Recombinant Monocyte-Derived Neutrophil Chemotactic Factor/IL-8," *The Journal of Immunology*, Vol. 144, 1990, pp. 2205-2210.

Applicant is enclosing Form PTO-1449 (one sheet), along with a copy of each listed reference for which a copy is required under 37 C.F.R. §1.98(a)(2). Pursuant to the undersigned attorney's obligation and duties under 37 C.F.R. §§ 1.56 and 1.98(a)(3) and (c), either English language abstracts, partial translations, or full translations are included for patent documents which are not in English for the express purpose of providing a concise explanation of the references to the Patent and Trademark Office with the opportunity to evaluate the same. Applicant respectfully requests the Examiner's consideration of the above reference(s) and entry thereof into the record of this application.

By submitting this Statement, Applicant is attempting to fully comply with the duty of candor and good faith mandated by 37 C.F.R. §1.56. As such, this Statement is not intended to constitute an admission that any of the enclosed references, or other information referred to therein, constitutes "prior art" or is otherwise "material to patentability," as that phrase is defined in 37 C.F.R. §1.56(a).

Applicant has calculated a processing fee in the amount of $180.00 to be due under 37 C.F.R. §1.17(p) in connection with the filing of this Information Disclosure Statement. Applicant has enclosed a check covering this fee, or authorized charging the fee to a deposit account or credit card, as indicated in the Transmittal accompanying this Information Disclosure Statement.

Respectfully submitted,

May 6, 2008
Date

/Richard G. Lione/
Richard G. Lione
(Reg. No. 19,795)

FORM PTO-1449		SERIAL NO. 10/579,323	CASE NO. 5404/152
LIST OF PATENTS AND PUBLICATIONS FOR APPLICANT'S INFORMATION DISCLOSURE STATEMENT		FILING DATE May 16, 2006	GROUP ART UNIT 1655
(use several sheets if necessary)	APPLICANT(S): Hideo Satsu et al.		CONFIRMATION NO. 6569

REFERENCE DESIGNATION **U.S. PATENT DOCUMENTS**

EXAMINER INITIAL		DOCUMENT NUMBER Number-Kind Code (If known)	DATE	NAME	CLASS/ SUBCLASS	FILING DATE
	C1	5,900,235	05/04/1999	Gosselin et al.		

FOREIGN PATENT DOCUMENTS

EXAMINER INITIAL		DOCUMENT NUMBER Number-Kind Code (If known)	DATE	COUNTRY	CLASS/ SUBCLASS	TRANSLATION YES OR NO
	C2	JP 2003-063991	03/05/2003	Japan		Abstract

EXAMINER INITIAL		OTHER ART – NON PATENT LITERATURE DOCUMENTS (Include name of author, title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date page(s), volume-issue number(s), publisher, city and/or country where published.)
	C3	Djeu, Julie Y., "Functional Activation of Human Neutrophils by Recombinant Monocyte-Derived Neutrophil Chemotactic Factor/IL-8," *The Journal of Immunology*, Vol. 144, 1990, pp. 2205-2210.

EXAMINER	DATE CONSIDERED

EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

PATENT ABSTRACTS OF JAPAN

(11)Publication number : 2003-063991
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(54) CYTOKINE INDUCING AGENT FOR USE IN ORAL ADMINISTRATION

(57)Abstract:
PROBLEM TO BE SOLVED: To provide a cytokine inducing agent for use in oral administration which is useful in treating various diseases such as infectious diseases, autoimmunity diseases, inflammations, tumors by which production of cytokines such as IFN-γ, IL-8 and the like in the living body can be promoted by oral administration thereof.
SOLUTION: A microorganism cell body, on the surface of which there is expressed cytokine inducing substances as to fusion protein, is used as cytokine inducing agent for use in oral administration. As to the above-mentioned microorganism, a lactobacillus such as Lactobacillus casei and the like may be used. The above-mentioned fusion proteins are the expressed products of DNA being combined in order with signal sequences, genes of cytokine inducing substances, and membrane anchor sequences. When a listeria hemolysin is used as to the gene of cytokine inducing substance, then IFN-γ can be induced, and when a flagellar protein flagellin of Simonella is used as to the gene of cytokine inducing substance, then IL-8 can be induced.

0022-1767/90/1445-2205$02.00/0
The Journal of Immunology
Vol. 144, 2205-2210, No. 6, March 15, 1990
Printed in U.S.A.

FUNCTIONAL ACTIVATION OF HUMAN NEUTROPHILS BY RECOMBINANT MONOCYTE-DERIVED NEUTROPHIL CHEMOTACTIC FACTOR/IL-8[1]

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Monocyte-derived neutrophil chemotactic factor (MDNCF)/IL-8, a novel cytokine, distinct from IL-1 and TNF was recently purified and cloned. This study was performed to investigate the biologic effect of recombinant MDNCF/IL-8 on human polymorphonuclear neutrophils (PMN) by assessment of their growth inhibitory activity against *Candida albicans*. The chemoattractant, FMLP was used as a positive control. We demonstrated that MDNCF/IL-8, similar to FMLP, effectively enhanced PMN-mediated anti-*Candida* activity. MDNCF/IL-8, from 1.0 to 1000 ng/mol, enhanced PMN-mediated anti-*Candida* activity, whereas FMLP was effective from 10^{-10} to 10^{-7} M. The optimal dose of MDNCF/IL-8 for PMN stimulation was 10 ng/ml which equalled the optimal chemoattractant dose. MDNCF/IL-8 itself, like FMLP, had no direct effect on *Candida* growth at any concentration and it stimulated antifungal activity only in PMN but not in monocytes. Interestingly, MDNCF/IL-8 failed to stimulate directly the production of superoxide from PMN or prime the respiratory burst of PMN exposed to FMLP. However, MDNCF/IL-8 was capable of releasing azurophilic enzymes from cytochalasin B-treated PMN into the extracellular space. Enhancement of PMN anti-*Candida* activity and release of azurophilic enzymes from PMN by MDNCF/IL-8 were inhibited in the presence of colchicine, which is a known inhibitor of degranulation. These results suggest that MDNCF/IL-8 induced antifungal action of PMN via oxygen-independent pathways. Furthermore, MDNCF/IL-8 induction of anti-*Candida* action by PMN was inhibited by pretreatment with *Bordetella pertussis* toxin, suggesting that enhancement of PMN antifungal activity by MDNCF/IL-8, as well as by FMLP, may be mediated by a GTP-binding protein.

PMN and monocyte/macrophages play a central role as effector cells against infiltrating microorganisms including *Candida albicans*. When an inflammatory reaction is induced by a pathogen, both series of phagocytes mobilize and accumulate at the inflammatory sites (1). This trafficking represents a response to numerous chemotactic stimuli that include bacterial chemotactic peptides (FMLP) and C5a formed by the C activation process (2, 3). The effector cells, in the inflammatory site, perform a complex set of functions, several of which contribute in the final stage for the removal of invading microorganisms. One important axis of this phagocytic cell response is the ability of monocytes/macrophages to interact with PMN to enhance their microbicidal function. The release of PMN-activating factors from monocytes/macrophages has been detected for some time but it is only recently that better purification methods and recombinant technology have provided the means to identify these molecules (4–7). A variety of cytokines including those released by mononuclear phagocytes, such as IL-1 and TNF, has recently been shown to be potent stimulators of several functional activities in PMN (8–18). The activities include phagocytosis, antimicrobial activity or antibody-dependent cellular cytotoxicity. Because some of these cytokines can augment the production of oxygen radicals and/or degranulation of PMN, it is believed that the enhancement of PMN function by monocyte-derived factors may be mediated via triggering of oxidative metabolism and/or degranulation of toxic nonoxidative metabolites in the environment of the organism or target cell.

Another cytokine, distinct from IL-1 and TNF, has recently been purified from the supernatant of human monocytes stimulated with LPS (19) that had potent chemotactic activity on PMN but not on monocytes, and its potency approached that of FMLP (19, 20). This MDNCF/IL-8 has been cloned and appears to have significant homology with several other proteins involved in the inflammatory response or tissue injury (21). A number of investigators have now also cloned MDNCF/IL-8 and related molecules not only from monocytes but also lymphocytes (22–26). MDNCF/IL-8 may therefore be partly responsible for PMN recruitment to sites of inflammation.

The goal of our study was to determine if rMDNCF/IL-8 had activities other than that of a chemoattractant. Our laboratory has, as a primary focus, been investigating the regulation of PMN function against *Candida albicans* by NK cells (27, 28) and cytokines (10). Using a rapid [^{3}H]glucose uptake assay developed in our labora-

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[1]This work was supported by a Grant RO1-AI-24699 from the National Institutes of Health.
[2]Address correspondence to Dr. Julie Y. Djeu, University of South Florida College of Medicine, Dept. of Medical Microbiology and Immunology, Box 10, 12901 Bruce B. Downs Blvd, Tampa, FL 33612.
[3]Abbreviations used in this paper: PMN, polymorphonuclear neutrophils; MDNCF, monocyte-derived neutrophil chemotactic factor; PT, pertussis toxin; LDH, lactate dehydrogenase.

tory. we have shown that human PMN were potent inhibitors of *Candida* growth in vitro (10, 27–29). In normal PMN, significant antifungal activity was usually detected even at an E/T ratio of 10/1. This activity could be further enhanced by IFN-γ and TNF (10), and these two factors could apparently work in synergy to activate PMN. We have now extended our study to show that MDNCF/IL-8 can also augment PMN function against *C. albicans*. The level of activation was compared to that of the standard PMN chemotactic agent, FMLP. Assessments of oxidative burst and degranulation were also performed to investigate whether enhancement of PMN antifungal activity by MDNCF/IL-8 was mediated via oxidative or nonoxidative pathways.

MATERIALS AND METHODS

Culture of C. albicans. C. albicans used in this study was a clinical isolate from a patient with chronic mucocutaneous candidiasis and identified according to the taxonomic criteria established by Ledder (30). The yeast was grown by biweekly transfer onto fresh Sabouraud's agar slants and incubation at 26°C. At the time of use, a small colony was obtained from the agar slant with a pipet and washed once with HBSS by centrifugation at 200 × *g* for 10 min.

Preparation of human PMN and mononuclear cells. Leukocyte buffy coats obtained from normal volunteers at the South West Florida Blood Bank were diluted 1/2 in HBSS and layered on 10 ml of Ficoll-Hypaque solution (Pharmacia Fine Chemicals, Piscataway, NJ). After centrifugation at 400 × *g* for 30 min at room temperature, the band of PBMC at the interphase was collected and washed twice with HBSS (10). The washed PBMC were resuspended to 1 × 10^6 to 1 × 10^6/ml in RPMI 1640 medium containing 10% fetal bovine serum, 2 mM L-glutamine, 100 μg/ml of penicillin/streptomycin, and 5 mM HEPES, which will simply be referred to as medium in the rest of the text. All media reagents were purchased from GIBCO, Grand Island, NY, and plastic supplies were from Costar, Cambridge, MA. Every precaution was taken in our laboratory to ensure that additives, medium, and the plasticware used in the course of the study were free of endotoxin as measured by the Limulus Amebocyte Lysate Test kit (Whittaker Bioproducts, Walkersville, MD) that has a sensitivity of 0.1 ng/ml.

For recovery of PMN, the white cell layer lying on the surface of the RSC pellet after Ficoll centrifugation was collected and lysed free of red blood cells by hypotonic shock with sterile distilled water for 30 s. The cells were washed twice in HBS before resuspension to the desired concentration. Cytocentrifuged preparations of PMN stained with Giemsa showed more than 98% PMN by morphology.

Assay for Candida growth inhibition. The optimal conditions for assessment of *C. albicans* growth inhibition were as previously described (29). Human PMN were diluted to 1 × 10^6, 3 × 10^6, and 1 × 10^7/ml in 1% FBS medium and 50 μl of each dilution was added to triplicate wells of a 96-well flat-bottomed microplate. For assessment of monocyte antifungal activity, PBMC were diluted to 3 × 10^6/ml, 1 × 10^6/ml, and 3 × 10^5/ml in 10% fetal bovine serum-medium after which 50 μl of PBMC were added to triplicate wells of a 96-cell plate tissue culture plate and allowed to adhere for 1 h at 37°C in a humidified 5% CO_2 in air atmosphere. Nonadherent cells were removed by vigorous washing three times with warm RPMI 1640 medium followed by addition of 50 μl of culture medium. Approximately 10% of the PBMC usually adhered to the well bottom and the adherent population consisted of more than 85% pure monocytes as judged by Wright-Giemsa stain. Trypan blue dye exclusion test for cell viability was also greater than 98%. After addition of PMN or monocytes to the wells, 50 μl of *C. albicans* at 1 × 10^4/ml in 1% FBS medium was added to all wells containing effector cells, yielding E/T ratios of 100/1, 30/1, 10/1, or 3/1. *Candida* were also added to empty wells to serve as controls. After the cell mixtures were incubated overnight at 37°C for 18 h, the medium in the wells was discarded and 50 μl of ^{3}H-glucose (NEC 607, D-[6,6-^{3}H]glucose, at sat. of 66.8 Ci/mmol, New England Nuclear, Boston, MA) diluted to 10 μCi/ml in sterile water was added. The water lysed the effector cells, leaving behind the mycelial colonies that had developed overnight in FBS. After an additional 3-h incubation at 37°C, the fungal growth in the wells was harvested by adding 50 μl of 3.25% sodium hypochlorite to all wells before collection with a MASH harvester.

The mean cpm of triplicate cultures was determined and the standard error was usually within 5% of the mean. Each experiment was repeated at least twice to ensure reproducibility of data. For clarity in presenting the data, the standard errors have been omitted. The percent growth inhibition of *Candida* was calculated as follows:

$$\% \text{ growth inhibition} = \frac{\text{cpm } \textit{Candida} \text{ alone} - \text{cpm effector/}\textit{Candida}}{\text{cpm } \textit{Candida} \text{ alone}} \times 100$$

Effect of rMDNCF/IL-8 and FMLP on PMN and monocyte antifungal activity. rMDNCF/IL-8 was expressed in *Escherichia coli* and purified to homogeneity using cDNA cloned from monocytes activated with LPS (21). The sp. act. of rIL-8 is estimated to be 2 × 10^8 U/mg in the neutrophil chemotactic assay and it is free of endotoxin as measured by the Limulus amebocyte lysate assay. FMLP was purchased from Sigma Chemical Co., St. Louis, MO. For activation of PMN, MDNCF/IL-8, and FMLP were serially diluted and added to PMN in the wells at a final concentration of 0.1 to 1000 ng/ml and 10^{-11} to 10^{-7} M, respectively. The preincubation of PMN with the above reagents was performed for 30 min at 37°C after which 50 μl of the *C. albicans* suspension was added. After an additional 16-h incubation at 37°C, the fungi were pulsed with ^{3}Hglucose for 3 h at 37°C, and were harvested as described. For activation of monocytes 10 ng/ml of MDNCF/IL-8, 10^{-8} M FMLP, or 100 U/ml of IFN-γ kindly provided by Genentech, Inc., San Francisco, CA) was added to wells containing adherent monocytes for 24 h at 37°C and the cells were washed before *C. albicans* was added for an additional 18-h incubation.

Assay of superoxide production from PMN. The total production of superoxide by PMN was measured by the method of ferricytochrome c reduction (34). A final concentration of 10 to 1000 ng/ml of MDNCF/IL-8 was added to 2 × 10^5 PMN in 1 ml of phenol red free HBSS containing 85 μM ferricytochrome c (type 3, Sigma). After incubation for 1 h at 37°C, the reaction was stopped by the addition of 800 U SOD (bovine E, Sigma). The cells were then centrifuged at 200 × *g* for 10 min at 4°C and absorbance of the supernatant was read at 550 nm in a spectrophotometer (Shimazu UV2100, Kyoto, Japan). The results were expressed as nanomoles of reduction of cytochrome c per 2 × 10^5 PMN using the extinction coefficient; E_{550} nm = 2.1 × 10^4 M^{-1}cm^{-1} (31).

Alternatively, the kinetics of superoxide production by PMN was measured. The assay mixture (1 ml), consisting of 2 × 10^6/ml of PMN in phenol red free HBSS containing 85 μm ferricytochrome c, was prewarmed for 5 min at 37°C in the thermostated chamber of the spectrophotometer. Then, 10^{-7} M FMLP or 1000 ng/ml of MDNCF/IL-8 was added and the absorbance changes were recorded continuously for 5 min. At the end of the 5 min incubation, 1000 ng/ml of MDNCF/IL-8 was added to the chamber containing FMLP whereas 10^{-7} M FMLP was added to that containing MDNCF/IL-8 to check for the priming effect of MDNCF/IL-8 and FMLP. The absorbance changes were then recorded for the next 15 min.

Exocytosis assay. Five hundred μl of PMN (3 × 10^6/ml) suspended in phenol red free HBSS were preincubated with or without 5 μg/ml of cytochalasin B (Sigma) at 37°C for 5 min. Exocytosis was then initiated by adding 10 to 1000 ng/ml of MDNCF/IL-8 or 10^{-7} M FMLP to the cells and incubating for another 30 min. The reaction was stopped by rapid cooling at 4°C followed by centrifugation. The enzymes, α-hexosaminidase, α-mannosidase, and β-glucuronidase, were determined in the cell-free media and cell pellets by incubating 75 μl of sample with 50-μl aliquots of the following substrates: 5 mM 4-methylumbelliferyl-*N*-acetylglucosamide in 0.2 M sodium citrate buffer (pH 4.5), 3 mM 4-methylumbelliferyl-*N*-α-*d*-mannoside in 0.2 M sodium acetate buffer (pH 4.5), and 5 mM 4-methylumbelliferyl-*N*-β-*d*-glucuronide in 0.2 M sodium acetate buffer (pH 4.5), respectively (32). Incubation was carried out at 37°C for 1 h and stopped by addition of 3 ml of 50 mM glycine buffer (pH 10.4). The 4-methylumbelliferon formed in the cell pellet or supernatant was measured fluorometrically in a Hitachi spectrofluorometer (Perkin-Elmer Corp., Norwalk, CT) and expressed in percent of total cellular content. Standard assays contained 2 or 4 nmol of 4-methylumbelliferone. Release of Lactate dehydrogenase (LDH) was measured using a commercial kit (LDH no. 500, Sigma).

Treatment with colchicine. Colchicine (Sigma) was added to the PMN suspension at a final concentration of 0.1 mM and the cells were incubated for 30 min at 37°C. Then *Candida* growth inhibition and exocytosis assays were carried out.

Treatment of pertussis toxin. Human PMN were incubated for 1 h at 37°C with a final concentration of 500 ng/ml of *Bordetella* PT, obtained from Sigma. To remove the toxin after treatment, PMN were centrifuged and washed three times before resuspending in FBS-medium for the *Candida* growth inhibition assay.

RESULTS

Effect of MDNCF/IL-8 and FMLP on PMN-mediated anti-Candida activity. The effect of MDNCF/IL-8 on PMN function was examined by addition of serial dilutions of MDNCF/IL-8 to PMN before *C. albicans* was added to yield E/T ratios of 100/1 to 10/1. MDNCF/IL-8 was also directly added to *C. albicans* alone to check whether it might have a direct effect on *Candida* growth. As shown in Figure 1, PMN in a healthy individual inhibited *Candida* growth in a dose-dependent manner from 60.3% at 100:1 to 12.1% at 10:1. In the presence of MDNCF/IL-8, an even greater inhibition of *Candida* by PMN was observed. MDNCF/IL-8, from 1.0 to 1000 ng/ml, was able to enhance PMN anti-*Candida* action and this enhancement was observed at all the ratios tested. Maximal effect of MDNCF/IL-8 was seen at the final concentration of 10 ng/ml. MDNCF/IL-8 itself had no direct effect on *Candida* growth at any concentrations used (see E/T ratio of 0/1). To determine whether FMLP, a known PMN stimulator and chemoattractant, could elicit a similar increase of PMN-antifungal activity, FMLP was added at a final concentration of 10^{-11} to 10^{-7} M to PMN. From 10^{-7} to 10^{-10} M, FMLP had a potent enhancing effect on PMN (Fig. 2). The optimal dose of FMLP for enhancing antifungal activity was 10^{-8} M, which is also the optimal dose established for its chemoattractant activity (33). The experiment was repeated with PMN from five healthy individuals and the results were very similar to those reported in Figures 1 and 2 (data not shown).

Effect of MDNCF/IL-8 on superoxide production from PMN. When PMN were incubated at 37°C for 1 h in the presence of 10 to 1000 ng/ml of MDNCF/IL-8, stimulation of ferricytochrome c reduction was not observed over control levels (Table I). As a positive control, superoxide production from PMN exposed to 10^{-7} M FMLP was also measured and was found to be significantly increased. To determine whether MDNCF/IL-8 was able to prime PMN for enhanced superoxide production induced by FMLP, PMN preincubated with 1000 ng/ml of MDNCF/IL-8 for 5 min at 37°C were then treated with FMLP at a final dose of 10^{-7} M. As a control, PMN treated with FMLP after preincubation with buffer was used. In addition, MDNCF/IL-8 was added after PMN had been preincubated with FMLP for 15 min at 37°C. Figure 3 shows that MDNCF/IL-8 was unable to alter the induction of superoxide production from PMN by FMLP, whether it was added before or after FMLP.

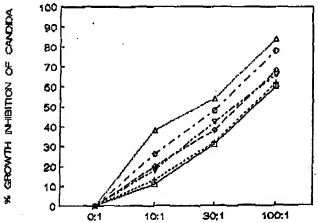

Figure 1. Effect of MDNCF/IL-8 on PMN antifungal activity. PMN were preincubated with 0.1 ng (+), 1.0 ng (○), 10 ng (△), 100 ng (◇), and 1000 ng (▽)/ml of MDNCF/IL-8 or medium (□) for 30 min and then *C. albicans* was added to the mixture, yielding the indicated E/T ratios. The mean of triplicate cultures is represented, and the SEM was within 5%.

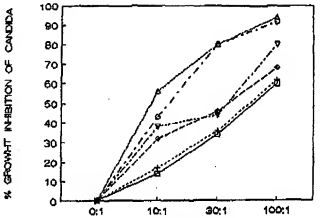

Figure 2. Effect of FMLP on PMN antifungal activity. Preincubation of PMN with 10^{-11} M (+), 10^{-10} M (○), 10^{-9} M (△), 10^{-8} M (◇) f, and 10^{-7} M (▽)ml of FMLP or medium (□) was performed for 30 min at 37°C after which *C. albicans* was added to the mixture. The mean of triplicate cultures is represented, and the SEM was within 5%.

TABLE I

Superoxide production from PMN exposed to MDNCF/IL-8 and FMLP[a]

Treatment of PMN	Superoxide Production nM/8 × 10^6 PMN/1 h
Control	15.2 ± 4.5[b]
MDNCF/IL-8 (ng/ml)	
10	14.8 ± 5.2
100	15.7 ± 3.7
1000	17.4 ± 4.8
FMLP (10^{-7} M)	43.5 ± 0.8

[a] PMN were treated with or without the indicated doses of MDNCF/IL-8 or FMLP and incubated for 1 h at 37°C.
[b] Each value shown represents the mean ± SD of three experiments.

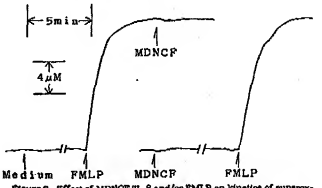

Figure 3. Effect of MDNCF/IL-8 and/or FMLP on kinetics of superoxide production from PMN. PMN were incubated with either 1000 ng/ml of MDNCF/IL-8 or 10^{-7} M FMLP for 15 min at 37°C after which either FMLP or MDNCF/IL-8 was added for another 15 min at 37°C.

Effect of MDNCF/IL-8 on exocytosis from PMN. Because activation of the PMN respiratory burst was not observed with MDNCF/IL-8, the effect of MDNCF/IL-8 on the release of α-hexosaminidase, α-mannosidase, and β-glucuronidase that are constituents of azurophil granules in PMN, was next examined. Exocytosis of these enzymes

from cytochalasin B-treated and untreated PMN was assessed after additional treatment with various doses of MDNCF/IL-8. Table II demonstrates that cytochalasin B-treated PMN, when incubated with 10 to 1000 ng/ml of MDNCF/IL-8 for 30 min at 37°C, released α-hexosaminidase, α-mannosidase, and β-glucuronidase. The positive control, FMLP, also potentiated exocytosis in cytochalasin B-treated PMN to a very high level. The release of cytosolic enzyme, LDH, was minimal, 4 ± 2% or 5 ± 2%, from PMN incubated with or without 1000 ng/ml MDNCF/IL-8 indicating that cell damage by MDNCF/IL-8 was negligible.

Effect of colchicine on MDNCF/IL-8 enhancement of PMN antifungal activity and exocytosis. Colchicine is known as an inhibitor of microtubule assembly as well as an inhibitor of release of lysosomal enzymes from PMN [34]. We therefore examined the possibility that colchicine might inhibit the action of MDNCF/IL-8 on PMN. As shown in Figure 4, the PMN antifungal activity induced by MDNCF/IL-8 was inhibited by the addition of colchicine. Similarly, FMLP enhancement of PMN anti-*Candida* activity was also inhibited by colchicine but to a lesser degree. Next, we examined whether colchicine might actually inhibit the release of lysosomal enzymes from PMN induced by MDNCF/IL-8 and FMLP. Table III demonstrated that PMN exocytosis activity induced by MDNCF/IL-8 and FMLP was also inhibited by addition of colchicine.

Effect of PT on MDNCF/IL-8 enhancement of PMN function. Studies of chemotactic factors, such as leukotriene B4, platelet-activating factor, C5a, and FMLP have suggested that occupancy of chemoattractant receptors is coupled with a PT-sensitive GTP binding protein (35). Accordingly, we raised the question whether activation of PMN by MDNCF/IL-8 might be regulated by such a protein. To investigate the role of GTP-binding protein in the regulation of PMN antifungal activity by MDNCF/IL-8, PMN were treated with PT before addition of MDNCF/IL-8. As a positive control, FMLP was used. When PMN were treated with PT, the ability of MDNCF/IL-8 to enhance PMN anti-*Candida* action was completely abrogated. Similarly, FMLP enhancement of anti-*Candida* function in PMN was also abrogated by PT (Fig. 5).

Effects of MDNCF/IL-8 on monocyte antifungal activity. To examine the effect of MDNCF/IL-8 on monocyte-mediated anti-*Candida* activity, 100 ng/ml of MDNCF/IL-8 was added to fresh monocytes for 24 h at 37°C before *C. albicans* was added to yield E/T ratios of 30/1 to 3/1. The incubation mixture of monocytes and *C. albicans* were further incubated for another 16 h before [^{3}H]glucose was added to measure radiolabel uptake by residual *Candida*. A representative experiment in Figure 6 shows that normal fresh monocytes inhibited *C. albicans*

TABLE II

Exocytosis by PMN induced by MDNCF/IL-8 and FMLP[a]

Chemotactic Factor	Cyt B	% release of α-Hexosaminidase	α-Mannosidase	β-Glucuronidase
Control	–	8.5 ± 0.9[b]	7.6 ± 1.0	4.8 ± 0.7
MDNCF/IL-8 (ng/ml)				
10	–	8.1 ± 0.8	7.7 ± 0.9	4.9 ± 0.5
100	–	8.4 ± 0.2	7.8 ± 0.9	4.7 ± 0.4
1000	–	8.8 ± 0.6	7.7 ± 0.6	5.0 ± 0.6
FMLP (10^{-7} M)	–	9.0 ± 0.5	8.0 ± 0.3	5.1 ± 0.3
Control	+	8.6 ± 0.3	7.8 ± 0.7	4.9 ± 0.6
MDNCF/IL-8 (ng/ml)				
10	+	8.7 ± 0.6	8.3 ± 1.0	5.8 ± 0.3
100	+	10.8 ± 0.9	9.6 ± 0.7	7.4 ± 0.9
1000	+	14.2 ± 0.4[c]	13.3 ± 0.4[c]	10.0 ± 0.8[c]
FMLP (10^{-7} M)	+	40.7 ± 1.1[c]	37.6 ± 0.8[c]	35.9 ± 0.8[c]

[a] PMN preincubated with or without cytochalasin B were exposed to the indicated dose of MDNCF/IL-8 or FMLP for 30 min and 37°C. Activities for each experiment were given as the percentage of total described enzymes.
[b] Each value shown represents the Mean ± SD of triplicate samples. Representative of at least three experiments performed as shown.
[c] Significant differences compared to control samples, ($p < 0.05$).

TABLE III

Effect of colchicine on MDNCF/IL-8- and FMLP-induced PMN exocytosis[a]

Chemotactic Factor	Colchicine	% Release of α-Hexosaminidase	α-Mannosidase	β-Glucuronidase
None	–	8.4 ± 0.6[b]	7.3 ± 0.5	5.0 ± 0.5
MDNCF/IL-8	–	13.7 ± 0.4[c]	13.6 ± 0.5[c]	9.7 ± 0.3[c]
FMLP	–	37.3 ± 0.9[c]	35.3 ± 0.7[c]	32.6 ± 0.5[c]
Control	+	8.6 ± 0.5	5.4 ± 0.4	3.9 ± 0.7
MDNCF/IL-8	+	7.2 ± 0.5	5.8 ± 0.8	4.3 ± 0.3
FMLP	+	18.3 ± 0.9[c]	15.8 ± 0.4[c]	11.8 ± 0.6[c]

[a] PMN preincubated with cytochalasin B were incubated with or without colchicine (0.1 mM) for 30 min at 37°C. Subsequently, PMN were stimulated with or without MDNCF/IL-8 (1000 ng/ml) or FMLP (10^{-7} M).
[b] Each value shown represents the Mean ± SD of triplicate samples. Representative of at least three experiments performed as shown.
[c] Significant differences compared to control samples, ($p < 0.05$).

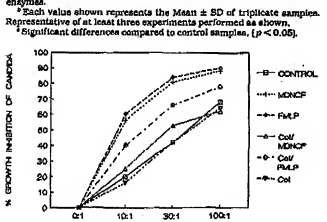

Figure 4. Effect of colchicine on MDNCF/IL-8 or FMLP-induced PMN antifungal activity. PMN were preincubated with 0.1 mM/ml of colchicine for 30 min at 37°C. Then 10 ng/ml of MDNCF/IL-8 or 10^{-8} M FMLP was added to the mixture and further incubated for 30 min before *C. albicans* was added. Untreated (control) PMN, MDNCF/IL-8 alone and FMLP alone served as controls. The mean of triplicate cultures is represented, and the SEM was within 5%.

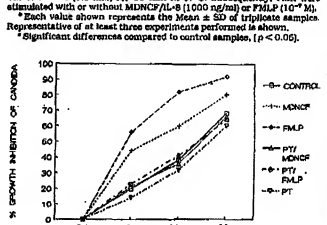

Figure 5. Effect of PT on MDNCF/IL-8 or FMLP-induced PMN antifungal activity. PMN, pretreated with PT, were stimulated with or without MDNCF/IL-8 (10 ng) or FMLP (10^{-8} M) and a further 30-min incubation was performed before addition of *C. albicans*. Untreated (control) PMN, MDNCF/IL-8 alone, and FMLP alone served as controls. The mean of triplicate cultures is represented, and the SEM was within 5%.

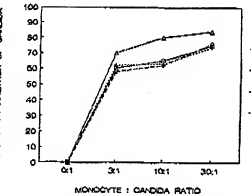

Figure 6. Effect of MDNCF/IL-8 or FMLP on monocyte-mediated *Candida* growth inhibition activity. Monocytes were preincubated with 100 ng/ml of MDNCF/IL-8, 10^{-8} M FMLP, or 100 U/ml of IFN-γ for 24 h and then *C. albicans* was added to the mixture yielding the indicated E/T ratios. After further culture for an additional 18 h, monocyte-mediated *Candida* growth inhibition activity was determined as described in *Materials and Methods*. The mean of triplicate cultures is represented, and the SEM was within 5%.

growth in a dose-dependent manner. In the presence of MDNCF/IL-8, *Candida* growth inhibitory activity by monocytes was not changed. In contrast, the control cytokine, IFN-γ, was effective in enhancing monocyte-mediated anti-*Candida* activity. Additionally, *Candida* growth inhibition by monocytes was not enhanced in the presence of 10^{-8} M FMLP.

DISCUSSION

Unlike monocytes, PMN are terminally differentiated phagocytes with a short half-life that are incapable of proliferation. However, PMN are endowed with other properties that are important to host defense (1). Their continued regeneration from the bone marrow and their abundant presence in the circulation make them available for a rapid acute response to invading microorganisms and to home quickly to the site of inflammation (3). Their mobility and function are affected by chemoattractants and cytokines released in the vicinity of the infection or inflammation (8–18). Our study shows that a chemoattractant released by monocytes and a number of other cell types, i.e., IL-8, can also act as a stimulator of biologic function in PMN.

Human PMN were found to readily respond to MDNCF/IL-8 by exhibiting a heightened ability to inhibit the growth of *C. albicans* in vitro. MDNCF/IL-8 at doses from 0.1 to 1000 ng/ml had no direct inhibitory effect on *C. albicans*. This observation is the first demonstration that MDNCF/IL-8 has the capability to activate PMN against a known opportunistic pathogenic fungus, suggesting the possibility that MDNCF/IL-8 may be important in PMN-antimicrobial response against other fungi and other types of microorganisms. The optimal dose of MDNCF/IL-8 that induced antifungal responses in PMN correlated with the dose of MDNCF yielding maximal chemotactic activity (19, 20). The low concentration of MDNCF/IL-8 (10 *ng/ml*) necessary for PMN activation suggests that monocytes may be able to release physiologic levels of factors that can effectively interact with PMN in vivo. The bacterial chemotactic product, FMLP, also enhanced PMN anti-*Candida* function. Thus, we provide clear evidence that chemoattractants can also serve as functional stimulators of PMN. In contrast, MDNCF/IL-8 did not stimulate monocyte anti-*Candida* activity, although the control cytokine, IFN-γ, was highly effective in this capacity. This is again consistent with earlier observations that MDNCF/IL-8 could not mobilize monocytes but only PMN (19).

It has been established that activation of the respiratory burst of PMN is of benefit in the antimicrobial process of PMN (36, 37). TNF and granulocyte-macrophage-CSF have the capability to induce or prime for a respiratory burst in PMN responding to FMLP, and to increase their exocytosis activity (8, 11–17). Therefore, we initially considered the mechanism of enhancement of PMN action against *Candida* by MDNCF/IL-8 to be dependent on oxygen intermediates. However, careful analysis showed that MDNCF/IL-8 failed to stimulate directly the production of superoxide from PMN or prime the respiratory burst of PMN exposed to FMLP.

Recent data have accumulated on the capability of PMN to use nonoxidative mechanisms to kill microorganisms, e.g., cationic proteins such as defensins and hydrolytic enzymes (38–40). Evidence for this pathway exists in vivo and in vitro. The catalase-positive fungus, *Candida parapsilosis*, that is readily killed by PMN from patients with myeloperoxidase-deficient and chronic granulomatous disease, is highly susceptible to azurophilic granule protein, cathepsin G (41, 42). Moreover, PMN from patients with chronic granulomatous disease also are able to normally eliminate catalase positive bacterial pathogen, *Neisseria gonorrhoeae* (43). We provide evidence here that MDNCF/IL-8 was capable of releasing azurophilic enzymes from cytochalasin B-treated PMN into the extracellular space, but not capable of inducing the respiratory burst from PMN. Furthermore, we showed that colchicine, which is a known inhibitor of microtubule assembly and of degranulation in PMN (34), was able to inhibit both MDNCF/IL-8-mediated enhancement of anti-*Candida* and exocytosis activity by PMN. Taken together, these results suggest that MDNCF/IL-8 mediated antifungal action by PMN is likely via oxygen-independent pathways. The alternative hypothesis that MDNCF might mediate antifungal activity in PMN via the induction of exocytosis of lysosomal enzymes is therefore favored.

Recent studies indicated that PMN-chemotactic agonists such as FMLP, C5a, platelet-activity factor and LTB4 may activate similar signal transduction systems which are dependent on a GTP-binding protein (35, 44, 45). The finding presented here showed that MDNCF/IL-8 as well as FMLP mediated PMN antifungal action was completely abrogated by PT, which binds GTP-binding proteins. Regulation of PMN anti-*Candida* function by MDNCF/IL-8 may therefore be via a PT-sensitive GTP-binding protein and the possibility exists that the MDNCF/IL-8 receptor is linked to a G protein that couples the receptor to an enzyme that degrades phosphoinositides.

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United States Patent [19]

Gosselin et al.

[11] Patent Number: 5,900,235

[45] Date of Patent: May 4, 1999

[54] **INTERLEUKIN-8 AS AN ANTIVIRAL AND ANTITUMOR AGENT**

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[21] Appl. No.: **08/674,633**

[22] Filed: **Jul. 5, 1996**

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[52] **U.S. Cl.** **424/85.2**; 424/85.1; 514/12; 514/13; 514/14; 530/324; 530/325; 530/326; 530/327; 930/141

[58] **Field of Search** 424/85.1, 85.2; 514/13, 14, 12; 530/324–327; 430/141

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[57] **ABSTRACT**

Interleukin-8 (IL-8) and analogs thereof is useful in the treatment of viral infections, caused by human and animal viruses, and cancers caused by oncoviruses.

15 Claims, 6 Drawing Sheets

Fig. 1A

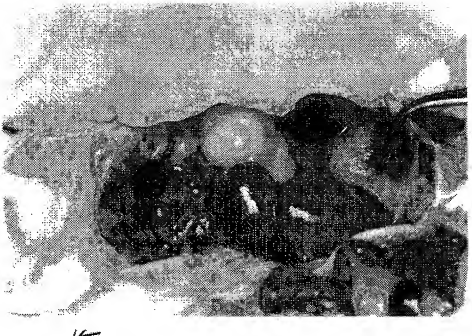


Fig. 1B

Fig. 1c

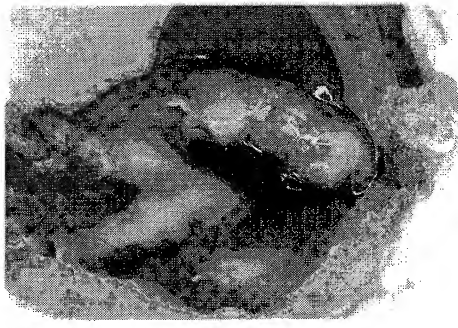


Fig. 1d

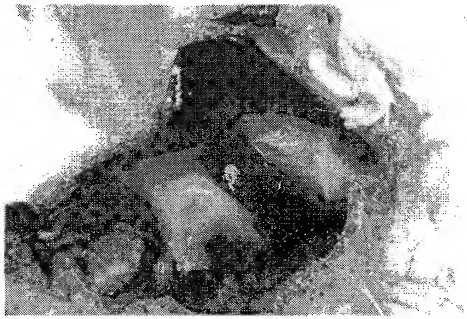
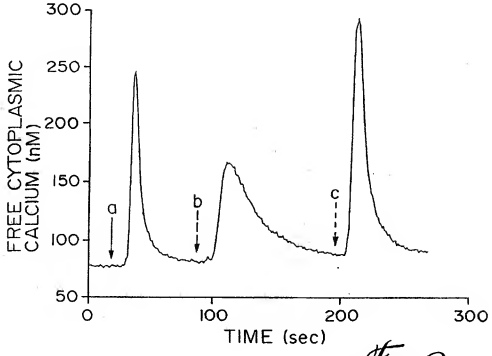


Fig. 1E

Fig. 1F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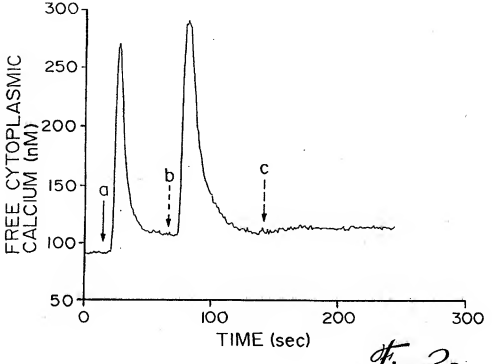
250
200
150
100
50
0
100
200
300
TIME (sec)
a
b
c

Fig. 2A


FREE CYTOPLASMIC CALCIUM (nM)
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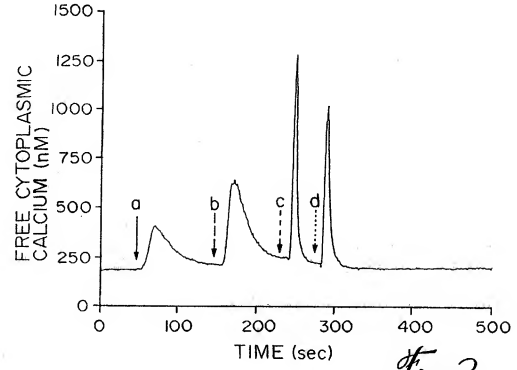
250
200
150
100
50
0
100
200
300
TIME (sec)
a
b
c

Fig. 2B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1250
1000
750
500
250
0
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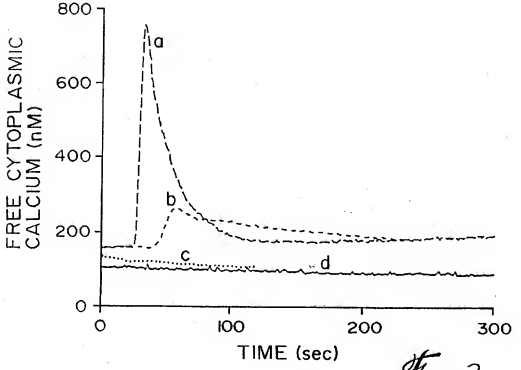
a
b
c
d
0
100
200
300
400
500
TIME (sec)

Fig. 2C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0
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a
b
c
d
0
100
200
300
TIME (sec)

Fig. 3A

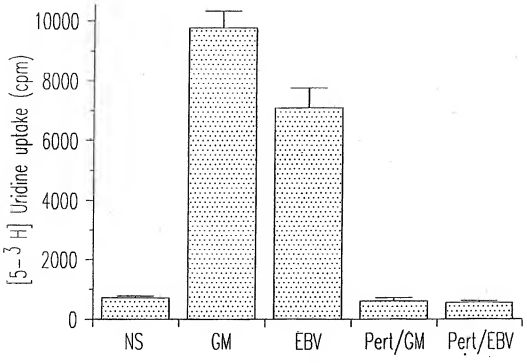
10000
8000
6000
4000
2000
0
[5-3 H] Uridine uptake (cpm)
NS
GM
EBV
Pert/GM
Pert/EBV

Fig. 3B

INTERLEUKIN-8 AS AN ANTIVIRAL AND ANTITUMOR AGENT

BACKGROUND OF THE INVENTION

a) Field of the Invention

The present invention is concerned with antiviral and anticancer activities of interleukin-8 (IL-8) and its use as a therapeutic agent in viral infections caused by human and animal viruses and in cancers caused by oncogene viruses.

b) Description of Prior Art

Many important infectious diseases afflicting mankind are caused by viruses of which some are frequently fatal; among such are rabies, smallpox, poliomyelitis, hepatitis, yellow fever, immune deficiencies and various encephalitic diseases. Others are significant in that they are highly contagious and create acute discomfort such as influenza, measles, mumps and chickenpox, as well as respiratory-gastrointestinal disorders. Others such as rubella and cytomegalovirus can cause congenital abnormalities; finally, there are viruses, known as oncoviruses, that can cause tumors and cancer in humans and animals.

Among viruses, the family of Herpesviridae is of great interest. Herpes viruses are highly disseminated in nature and highly pathogenic for man. For example, Epstein-Barr virus (EBV) is known to cause infectious mononucleosis in late childhood or adolescence or in young adults. The hallmarks of acute infectious mononucleosis are sore throat, fever, headache, lymphadenopathy, enlarged tonsils and atypical, dividing lymphocytes in the peripheral blood. Other manifestations frequently include mild hepatitis, splenomegaly and cerebritis. EBV is also associated with two forms of cancer: Burkitt's lymphoma (BL) and the nasopharyngeal carcinoma (NPC). In endemic areas of equatorial Africa, BL is the most common childhood malignancy, accounting for approximately 80% of cancers in children. While moderately observed in North American Caucasians, NPC is one of the most common cancers in Southern China with age incidence of 25 to 55 years. EBV, like the cytomegalovirus, is also associated with post-transplant lymphoproliferative disease, which is a potentially fatal complication of chronic immunosuppression following solid organ or bone marrow transplantation.

Another herpes virus, namely Herpes Simplex type 1 (HSV-1) is implicated as the etiologic agent of gingivostomatitis. Manifestations are fever, sore throat, and ulcerative and vesicular lesions in the mouth. The most severe clinical state caused by HSV is the primary genital herpetic infection. While HSV-1 can cause genital herpetic infection, HSV-2 is the main virus associated with this disease. This HSV infection is accompanied by vesicles, pustules and ulcers causing lesions on genital parts. A urinary retention syndrome may also be encountered. More than 80% of people are seropositive to HSV-1 or HSV-2 and studies have indicated a frequency of recurrence or viral reactivation as high as 60%. Other diseases are also associated with HSV including skin and eye infections, for example, chorioretinitis or keratoconjunctivitis. Approximately 300,000 cases of HSV infections of the eye are diagnosed yearly in the United States of America.

Human Herpes virus-6 (HHV-6) has a marked tropism for cells of the immune system and therefore, HHV-6 infection may result in alteration of the immune response. It is now clear that HHV-6 is the cause of exanthem subitum as a primary infection in children. Recent studies indicate that a significant proportion of organ transplant recipients who are seropositive before transplantation, demonstrate serologic evidence of reactivation subsequent to immunosuppression. Heterophil-negative mononucleosis-like illness and non-A, non-B hepatitis also have been associated with active HHV-6 infection. HHV-6 has often been isolated from patients with human immunodeficiency virus (HIV-1) infections. The fact that HIV and HHV-6 can reside in the same target cell has led to speculation that HHV-6 infection may act as a cofactor in the progression of HIV-seropositive patients to symptomatic AIDS. Recent studies also suggest that a human herpes virus is closely associated with HIV diseases. In fact, Kaposi sarcoma (KS), a neoplasm occurring mainly in HIV-infected person, was found to have an infectious etiology. While the virus has been named KS-associated herpesvirus, its formal classification is likely to be HHV-8.

In all infectious diseases, the efficacy of therapy often depends on host immune response. This is particularly true for herpes viruses; indeed, the ability of all herpes viruses to establish latent infections results in an extremely high incidence of reactivated infection in immunocompromised patients. In renal transplant recipients, 40% to 70% reactivate latent HSV infections, and 80% to 100% reactivate CMV infections. Such viral reactivations have also been observed in HIV-positive patients (AIDS).

Today, the number of therapeutic agents available for the treatment of viral infections remains relatively limited. For example, four major compounds are mainly used in the treatment of herpes virus infections: idoxuridine, vidarabine, acyclovir and ganciclovir. Their efficacy is limited and they cause many side effects. Allergic effects have been reported in 35% of patients treated with idoxuridine which is used only to treat HSV infection of the eye. The most common side effects of vidarabine are gastrointestinal disturbances (15% of patients). The major side effect of acyclovir is the alteration of renal function; and because acyclovir is a nucleoside analog that can be incorporated in both viral and the host cell DNA, normal division of the host cell can be affected. Regarding ganciclovir, the most important side effects are neutropenia and thrombocytopenia that occur in about 40% of AIDS patients.

Thus, there is an urgent need for the development of efficacious therapy for the treatment of viral infections.

The accumulation of leukocytes in diseased tissues is recognized as a hallmark of the inflammatory process. Recruitment of leukocytes at inflammatory sites is triggered by the local production of chemotactic cytokines. Proteins that exhibit such properties have been classified in two subfamilies according to the position of the first two cysteines, which either are separated by one amino acid (CXC proteins) or are adjacent (CC proteins). The members of the two subfamilies differ in their target cell selectivity and the chromosomal location of their genes (review by Baggiolini, et al., Adv. Immunol. 55: 97, 1994). Among the chemotactic cytokines, interleukin-8 (IL-8), which belongs to the CXC family, was originally identified in the culture supernatants of stimulated human blood monocytes. IL-8 is a nonglycosylated protein synthesized as a precursor of 99 amino acids and secreted after cleavage of a sequence of 20 residues. The mature molecule formed has 79 residues and is processed proteolytically at the N-terminus, yielding the predominant form of 72 amino acids with a molecular weight of about 8383. Structure, sequence and biological properties of IL-8 have been reviewed by Baggiolini M. et al. (Adv. Immunol. 55: 97, 1994).

IL-8 is produced by many cells such as keratinocytes, epithelial cells, synoviocytes and hepatocytes, to name a

few. Among peripheral blood leukocytes, monocytes and neutrophils rather than lymphocytes were found to be the major cellular sources of IL-8.

IL-8 exerts many biological activities in vitro and in vivo. IL-8 is well known for its chemotactic activity and its ability to cause degranulation of human neutrophils. Shape change, activation of the motile system and a rise in cytosolic free $Ca^{2+}$are rapidly detected in neutrophils treated with IL-8. The release of vitamin B_{12}-binding protein from specific granules was also observed. IL-8 also causes degranulation of the azurophil granules and release of elastase and other hydrolases. Such degranulation is accompanied by the upregulation of a variety of adhesion molecules at the cell membrane. Degranulation also results in the enhanced expression of the complement receptor type I (CR1) and III (CR3). IL-8 is also a chemotactic factor for eosinophils and for human lymphocytes, particularly T cells.

The biological effects of IL-8 are mediated through seven transmembrane domain, G-protein-coupled receptors. Two types of IL-8 receptors have been described and are defined as the type A and type B. IL-8 receptor type A has a high affinity for IL-8 and a low affinity for Groα(melanoma growth stimulating activity), whereas type B has high affinity for both cytokines. While among peripheral blood leukocytes, neutrophils strongly express both types of IL-8 receptors, monocytes and $CD8^+$ T lymphocytes expressed IL-8 receptors to a lower level. No detectable level of IL-8 receptors was found on B cells and $CD4^+$ T lymphocytes.

There is accumulating evidence in support that IL-8 plays an important role in the inflammatory process of many pathologies; indeed, IL-8 has been detected in inflammatory tissues or exudates such as in psoriatic scale extracts, in synovial fluid from patients with rheumatoid arthritis or gout, in pleural fluid from emphysema patients, and in bronchoalveolar lavages from patients with respiratory distress syndrome. Moreover, antiviral properties have recently been ascribed to the chemokines RANTES and MIP-1α and β, belonging to the C—C chemokine subfamily, which were found to induce inhibition of HIV-1, HIV-2 and SIV replication in vitro

Furthermore, IL-8 has been recently reported to have an antiviral effect (Mackewicz and Levy, 1992 AIDS Research and Human Retrovirus 8: 1039–1050). It was shown that IL-8 can inhibit HIV replication in CD4+ lymphocytes under specific conditions. In fact, IL-8 was found to affect viral replication of naturally infected CD4+ cells but had no effect on acutely infected cells. There are several putative mechanisms of antiviral activity; some may be highly selective as sould be the blockade of the attachment of a specific virus to its target cell. Indeed, Idoxuridine, Vidarabine, Acyclovir and Gangiclovir which are specifically used in the treatment of Herpes virus infection, have no effect on other viruses such as HIV, and thus constitute examples of drugs with selective antiviral activity.

Accordingly, data presented by Mackowicz and Levy in no way make predictable that IL-8 might show antiviral activity against all viruses. The selectivity for specific viruses of most drugs presently used in the treatment of viral infection strongly supports this statement. Therefore, the report of the antiviral effect of IL-8 on HIV does not allow any conclusion on the general antiviral activity of IL-8. To date, there is no other proven report of antiviral activity of IL-8.

Finally, TALMADGE'S PCT application PCT/US95/12099 published Mar. 28, 1996 under the publication number WO 96/09062 describes polypeptide analogs of IL-8 and claims that the analogs can be used for the treatment of pathological conditions such as viral infection, bacterial infection, fungal infections, yeast infection, parasitic infection among others. However, TALMADGE doe not present any data to prove its claim which is based on extrapolation or mere desire for the polypeptide to do so.

It would be highly desirable to provide an antiviral agent with greater efficacy and which would not present the undesirable side effects of the known antiviral agents.

SUMMARY OF THE INVENTION

The present invention provides methods for the prophylaxis or treatment of viral infections and cancer caused by oncoviruses.

In accordance with these methods, a pharmaceutically or physiologically acceptable, therapeutically effective amount of an interleukin-8 agent is administered to a human or animal in need of such treatment.

One aim of the present invention is to provide an antiviral agent and method which would be more efficacious for the prophylaxis and treatment of viral infections and which would not present the undesirable side effects of the known antiviral agents.

Another aim of the present invention is to provide an antiviral agent and method for the prophylaxis or treatment of cancers induced by oncoviruses such as retroviruses, papillomaviruses, adenoviruses and herpesviruses.

Another aim of the present invention is to provide an antiviral agent and method for the prophylaxis or treatment of viral infections in immunosuppressed patients and animals.

In accordance with the present invention, there is provided the use of IL-8 agent as an antiviral agent against herpes viruses selected from the group consisting of EBV, HSV-1, HSV-2, CMV, VZV, HHV-6, HHV-7 and HHV-8; and in a method for the prophylaxis or treatment of infections caused by such herpes viruses.

In accordance with the present invention, there is also provided the use of IL-8 agent as an antiviral agent against other human and animal viruses, including, but not limited to, porcine enteroviruses belonging to the picornaviridae or bovine diarrhea virus belonging to the togaviridae family, or bovine respiratory syncytial virus belonging to the paramyxoviridae.

In accordance with the present invention, there is also provided the use of IL-8 agent as an antiviral agent in the treatment of viral infections in humans and animals in association with other antiviral agents, including but not limited to interferon-α, -β, -γ, tumor necrosis factor α, ganciclovir, acyclovir, vidarabine, idoxuridine, and prostaglandins or prostaglandin analogs.

In accordance with the present invention, there is also provided the use of IL-8 agent as an antiviral agent for the prophylaxis and treatment of cancers induced by oncoviruses such as retroviruses, papillomaviruses, adenoviruses and herpesviruses.

In accordance with the present invention, there is also provided the use of IL-8 agent as an antiviral agent against cancers induced by oncoviruses in association with other anticancer agents including but not limited to adriamycine, cyclophosphamide and methotrexate.

In accordance with the present invention, there is also provided the use of IL-8 agent as an antiviral agent for the prophylaxis and treatment of viral infections in immunosuppressed patients and animals.

Immunosuppressed patients include patients who have undergone organ or tissue transplantation and are treated with immunosuppressive agents including but not limited to azathioprine, corticosteroids, adriamycine, cyclophosphamide and methotrexate. Immunosuppressed patients also include patients with any form of cancer or neoplasic diseases treated or not with anticancer chemotherapeutic agents including but not limited to adriamycine, cyclophosphamide and methotrexate. Immunosuppressed patients also include patients with inflammatory diseases treated with antiinflammatory agents including but not limited to corticosteroids, methotrexate, azathioprine and cyclophosphamide. Immunosuppressed patients also include patients with shock or severe trauma including but not limited to burn injury, or patients undergoing chronic hemodialysis.

In accordance with the present invention, there is also provided the use of IL-8 agent as an antiviral agent against viral infections in immunosuppressed patients and animals in association with other antiviral agents.

BRIEF DESCRIPTION OF THE DRAWINGS

The file of this patent contains at least one drawing executed in color: Copies of this patent with color drawing (s) will be provided by the Patent Trademark Office upon request and payment of the necessary fee.

FIGS. 1*a* to 1*f* illustrate the inhibitory effect of IL-8 on EBV-induced tumor growth and splenomegaly in SCID mice.

FIGS. 2*a*, 2*b* and 2*c* illustrate the effects of IL-8 and Groεon EBV-induced Ca^{2+} mobilization in human neutrophils.

FIGS. 3*a* and 3*b* illustrate the effects of pertussis toxin on EBV-induced Ca^{2+} mobilization (A) and RNA synthesis (B) in human neutrophils.

DESCRIPTION OF PREFERRED EMBODIMENTS WITH REFERENCE TO THE DRAWINGS i) IL-8 Agent

The IL-8 agent of the present invention is interleukin-8 in the 72 amino acid form or the 77 amino acid form, derivatives or analogs of interleukin-8. It is known in the art that minor addition or deletion of amino acids to IL-8 may slightly alter, viz. enhancing or decreasing the activity of IL-8. Accordingly, derivatives and analogs of IL-8 also includes modified peptides showing significant biological activity analogous to that of IL-8 in various biological systems. Significant biological activity in the context of the invention includes but is not limited to the activation of human neutrophils and thus also includes an antiviral activity similar to that of IL-8.

The term IL-8 agent also includes antibodies to the IL-8 receptor, or anti-idiotypic antibodies to antibodies raised against IL-8 or one of the above-mentioned analogs or variants of IL-8, which elicit an IL-8-like biological response, such as an antiviral effect.

The term IL-8 agent also includes the IL-8 receptors (types A and B) or other receptors which bind IL-8, or peptides corresponding to selected regions of these receptors or proteins (or glycoproteins or lipoproteins) of the viral envelope or peptides corresponding to selected regions of these proteins, which prevent the binding of viral particles to the IL-8 receptors or to other receptors which bind IL-8.

The term IL-8 agent also includes analogs or variants of the IL-8 receptors (or of other receptors which bind IL-8); analogs or variants of peptides corresponding to selected regions of these receptors; and proteins (or glycoproteins or lipoproteins) of the viral envelope, of peptides corresponding to selected regions of these proteins.

The term IL-8 agent however does not include peptides of about 17 amino acids having the following formula:

Glu-Leu-Arg-Cys-Xaa_1-Cys Xaa_2-Xaa_3-Xaa_4-Xaa_5-Xaa_6-Xaa_7-Xaa_8-Xaa_9-Xaa_{10}-Xaa_{11}-Xaa_{12}

wherein:

Xaa_1 is Gln, Met, or Val;
Xaa_2 is Ile, or Val;
Xaa_3 is Lys, Gln, or Ser;
Xaa_4 is Thr, or Ile;
Xaa_5 is Tyr, Leu, Met, His, Val, or Thr;
Xaa_6 is Ser, Gln, Thr, or Ala;
Xaa_7 is Lys, Arg, or His;
Xaa_8 is absent or is Pro, Phe, or Gly;
Xaa_9 is absent or is Phe, Ile, or Val;
Xaa_{10} is absent or is His, Lys, or Arg;
Xaa_{11} is absent or is Pro, Leu, or Phe; and
Xaa_{12} is absent or is Lys, His or Arg.

ii) Viral infections

The viral infections which may be treated with the IL-8 agent, in accordance with the invention, are infections caused by human and animal viruses.

The expression "human and animal viruses" is intended to include, without limitation, DNA and RNA viruses in general and Retroviridae. DNA viruses include parvoviridae, papovaviridae, adenoviridae, herpesviridae, poxviridae and hepadnaviridae. RNA viruses include picornaviridae, togaviridae, orthomyxoviridae, paramyxoviridae, coronaviridae, reoviridae, oncornaviridae and filoviridae.

The antiviral activity of IL-8 against the Epstein-Barr virus (EBV) has been investigated in vivo. It is well-known that SCID mice, characterized by the absence of B and T lymphocytes, often develop B lymphomas when reconstituted with peripheral blood lymphocytes from EBV-infected donors. This infection is accompanied by splenomegaly, enlargement of the peritoneal cavity and diarrhea. Thus, SCID mice represent an interesting in vivo model of human lymphomagenesis involving viral pathogens. To evaluate the antiviral activities of IL-8, EBV-transformed B cells (B95-8 cell line) were injected intraperitoneally into SCID mice; IL-8 was then administered intraperitoneally in different schedules in accordance with Table 1 below.

TABLE 1

Protocol of the in vivo study of the effects of IL-8 on EBV-infected SCID mice

Group 1	Untreated, animals
Group 2	Injection of IL-8 on day 0
Group 3	Injection of B95-8 cells on day 0
Group 4	Injection of B95-8 cells and of IL-8 on day 0 at 30 min interval
Group 5	Injection of B95-8 cells on day 0 and of IL-8 on days 0, 1, 2, 3, 4, 5
Group 6	Injection of B95-8 cells on day 0 and of IL-8 on days 0, 7, 15, 21, 28
Groups 1–6	Animals sacrificed and autopsied at day 49

EBV-infected B95-8 cells (60×10^6) were injected intraperitoneally (IP) into SCID mice. At the indicated times, mice were treated with recombinant IL-8. After 49 days, mice were sacrificed and autopsied. Each group consisted of 3 mice. IL-8 was always administered as a 25 μg bolus (IP) per mouse, in solution in NaCl 0.9% containing 0.01% BSA.

At four weeks post-infection, mice treated with B95-8 cells alone showed a significant inflammation of the peri-

toneal cavity as assessed by the occurrence of abdominal swelling, and had diarrhea, a characteristic of tumor growth in this model. In contrast, such symptoms were not observed in mice treated 5 times with 25 μg of IL-8 at one week intervals (group 6).

In mice treated with 25 μg IL-8 for 6 consecutive days (group 5), the diarrhea was observed at four weeks post infection but was less severe than in infected untreated animals (group 3).

In mice treated only once with 25 μg IL-8 (group 2), the peritoneal inflammation and diarrhea observed at four weeks post infection was as severe as in infected untreated animals of group 3.

At four weeks, animals of groups 1, 2 and 6 appeared normal and did not show the above-mentioned symptoms. Animals of group 3 died in the course of the 5th and 6th weeks post infection; autopsy revealed massive hemorrhage at the level of spleen and liver. At seven weeks post infection, surviving animals of all groups were sacrificed and autopsied.

Infected animals treated once with IL-8 (group 4) showed marked splenomegaly and a tumor of approximately 0.7 cm diameter was found in each mouse, as shown in FIG. 1b. In non-infected, untreated animals (group 1), spleens were normal and the tumor was absent as shown in FIG. 1a. In animals treated six consecutive days with 25 μg IL-8 (group 5), splenomegaly and the tumor were observed but were reduced in comparison to animals of group 4. In animals treated four times with 25 μg IL-8 at one week intervals (group 6), tumors were not detectable and spleens (and other organs) were morphologically undistinguishable from those of uninfected animals (groups 1 and 2).

EBV has recently been shown to interact with human neutrophils in vitro and to modulate RNA and protein synthesis. IL-8 is known to induce shape change and a transient rise of the intracellular free Ca^{2+0}concentration ($[Ca^{2+}]i$) in human neutrophils. We have observed that EBV induces a similar but not identical (different kinetic of Ca^{2+} accumulation) effect on $[Ca^{2+}]i$ in isolated human neutrophils. We thus investigated the effect of IL-8 on EBV-induced rise in $[Ca^{2+}]i$ in neutrophils. Ca^{2+} mobilization was monitored in human neutrophils loaded with the fluorescent probe FURA-2/AM and treated with IL-8 or Groεprior to or after exposure to EBV. The rise of $[Ca^{2+}]i$ induced by EBV was strongly inhibited when neutrophils were pretreated with IL-8. On the other hand, EBV pretreatment of neutrophils did not suppress the effect of IL-8 or Groα(FIG. 2a, 2b, 2c). Since the IL-8 receptor is a G-protein-coupled receptor, the inventors next examined the potential involvement of G-protein in EBV-induced Ca^{2+} mobilization and RNA synthesis by pretreating neutrophils with pertussis toxin, a known inhibitor of guanine nucleotide-binding regulatory proteins. The results obtained show that pertussis toxin significantly inhibits Ca^{2+} mobilization and RNA synthesis induced by EBV, suggesting that the EBV-induced events are G-protein-mediated.

While the mechanism involved in the antiviral and/or antitumoral effect of IL-8 is not clear, it can be hypothesized that IL-8 triggers or amplifies natural antiviral mechanisms, such as the production of tumor necrosis factor alpha (TNFα) or interferons. Alternatively IL-8 might block the putative inhibition of natural antiviral mechanisms induced by EBV. Furthermore, given the observed effect of IL-8 on EBV-induced biological effects on isolated neutrophils (FIGS. 2 and 3), it is also possible that EBV interaction with its target cells involves an IL-8 receptor (or binding sites that can recognize IL-8), in which case IL-8 might prevent the virus-target cell interaction either through competition for the binding site (steric hindrance) or downregulation of receptor expression. Alternatively, exposure of target cells to IL-8 might downregulate receptor functionality through uncoupling of the virus receptor from signal transduction mechanism involved in the process of viral infection including attachment, internalization, replication and downregulation of natural cellular antiviral mechanisms. Additional support for such hypothesis comes from the recent report that chemokine receptor-related proteins are encoded by various viruses and expressed on plasma membrane of infected cells. In the EBV system, it is known that the virus induces BLR-1 (probably identical to EBI-2) and BLR-2 (identical to EBI-1) receptors in transformed cells, which are G-protein-coupled receptors highly homologous to the IL-8 receptor. The expression of these cytokine receptors homologous to IL-8 receptors on EBV-infected cells may render the infected cells, the B cells which do not carry IL-8 receptors, susceptible to a putative antiviral activity of IL-8. These hypotheses are given for the purpose of providing some insights on putative mechanism(s) of the antiviral activity of IL-8 and should not limit the scope of the invention.

These results indicate that IL-8 can be useful in the treatment of viral infections and cancers caused by oncogene viruses.

iii) Dose Ranges

The magnitude of therapeutic dose of an interleukin-8 agent will vary with the nature or the severity of the condition to be treated, with the particular IL-8 agent, with the concommitant use of other active compounds and its route of administration and will be determined based on clinical judgement. It will also vary according to the age, weight and response of the individual patient. An effective dosage amount of the IL-8 agent can thus be determined by pharmacokinetic studies by the clinician after a consideration of all the criteria and use of best judgment on the patient's behalf. In a general manner, the effective dosage is of about 1 to 10 nM in the blood. Accordingly, the clinician will administer a dosage that will produce such concentration in the blood.

iv) Pharmaceutical Compositions

Any suitable route of administration may be employed for providing a mammal, especially a human with an effective dosage of an IL-8 agent of the present invention. For example, the dosage may be administered orally, parenterally, topically, intraarterially, intraperitoneally, intravenously, intrapleurally, intraoccularly, by injection, subcutaneously or the like. It is understood that injection comprises also perfusion and continuous infusion. Dosage forms include tablets, capsules, powders, solutions, dispersions, suspensions, crams, ointments and aerosols.

The pharmaceutical compositions of the present invention comprise an IL-8 agent as an active ingredient, and a pharmaceutically acceptable carrier and optionally other therapeutic ingredients.

The compositions include compositions suitable for oral or parenteral administration. Conveniently they are presented in unit dosage form and prepared by any of the methods well-known in the art of pharmacy. The unit dosage form may be a slow released unit dosage form.

In practical use, the IL-8 agent can be combined as the active ingredient in intimate admixture with a pharmaceutical carrier according to conventional pharmaceutical compounding techniques. The carrier may take a wide variety of forms depending on the form of preparation desired for administration. In preparing the compositions for oral dos-

age form, any of the usual pharmaceutical media may be employed, such as, for example, water, glycols, oils, alcohols, flavoring agents, preservatives, coloring agents and the like in the case of oral liquid preparations, such as, for example, suspensions, elixirs and solutions; or carriers such as starches, sugars, microcrystalline cellulose, diluents, granulating agents, lubricants, binders, disintegrating agents and the like in the case of oral solid preparations such as, for example, powders, capsules and tablets. Because of their ease of administration, tablets and capsules represent the most advantageous oral dosage unit form in which case solid pharmaceutical carriers are employed. If desired, tablets may be coated by standard aqueous or nonaqueous techniques.

Pharmaceutical compositions of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the IL-8 agent, as a powder or granules or as a solution or a suspension in an aqueous liquid, a non-aqueous liquid, an oil-in-water emulsion or a water-in-oil emulsion. Such compositions may be prepared by any of the methods of pharmacy such methods including the step of bringing the IL-8 agent into association with the carrier which includes one or more necessary ingredients. In general, the compositions are prepared by uniformly and intimately admixing the IL-8 agent with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product into the desired presentation. For example, a tablet may be prepared by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine, the active ingredient in a free-flowing form such as powder or granules, optionally mixed with a binder, lubricant, inert diluent, surface active or dispersing agent. Molded tablets may be made by molding in a suitable machine, a mixture of the powdered compound moistened with an inert liquid diluent.

It will be understood that the interleukin-8 agent is to be administered in pharmacologically or physiologically acceptable amounts, by which is to be understood amounts not harmful to the patient, or amounts where any harmful side effects in individual patients are outweighed by the benefits. Similarly, the interleukin-8 agent is to be administered in a therapeutically effective amount, which is to be understood is an amount meeting the intended therapeutic objectives, and providing the benefits available from administration of interleukin-8 agent.

EXAMPLES

In Vivo Studies

Example 1 Antiviral effect of IL-8 on EBV-infected SCID mice in vivo.

Female SCID-CB17 mice (Charles River, St. Constant, Canada) aged 8 weeks were used in this study. Six groups of three mice each were formed, infected with EBV-transformed cells and treated or not with IL-8, as summarized in Table 1 above (groups 3 to 6). At day 0, B95-8 cells (60×10^6) were injected intraperitoneally (IP) into SCID mice. When indicated, mice also received one or more injections (IP) of IL-8 (groups 2, 4 to 6) (25 μg/mouse/injection) (Peprotech Inc., Rocky Hid, N.J.). The IL-8 used was the 72 amino acid form and was used in solution in NaCl 0.9% containing 0.01% BSA. After seven weeks of treatment, mice were killed by cervical dislocation and autopsied. Tumors and other tissues of interest were photographed (FIGS. 1*a* to 1*f*), dissected and kept frozen for immunofluorescence studies and for viral DNA analysis. FIG. 1*a* illustrates non infected untreated mice (group 1). FIGS. 1*b* and 1*c* illustrate EBV-infected mice treated with one injection of IL-8 30 min after infection with B95-8 cells (group 4). FIGS. 1B and 1C show the abdominal cavity of the same animal, FIG. 1C being a close-up photography (group 4). FIGS. 1*d* and 1*e* illustrate EBV-infected mice treated weekly with IL-8 during four successive weeks beginning 30 min after infection with B95-8 cells (group 6). FIG. 1*f* illustrates abdominal cavity of EBV-infected mice treated once with IL-8 (left), four times with IL-8 (middle) and untreated (right).

In Vitro Studies

Example 2

Inhibitory Effect of IL-8 (and Groα) on Calcium Mobilization Induced by EBV in Isolated Human Neutrophils

Neutrophil suspensions (10×10^6 cells/ml) were incubated with the fluorescent probe Fura-2/acetoxymethyl ester (Molecular Probe, Eugene, Oreg.) (1 μM for 30 min at 37° C.). The cells were then washed free of the extracellular probe and resuspended in Hank's balanced salt solution (HBSS) containing 1.6 mM calcium and supplemented with 10 mM HEPES. Suspensions of Fura-2-loaded cells were treated with EBV (10^6 transforming units [TFU]/ml) and with IL-8 or Groα(100 nM) in different sequences. Fluorescence (FIGS. 2*a*, 2*b* and 2*c*) was monitored (excitation and emission wavelengths, 340 and 510 nm, respectively) using an Aminco-Bowman, series 2 Apparatus (SLM-Aminco, Rochester, N.Y.). Viral preparations of EBV strain B95-8 were produced as previously described. Briefly, B95-8, which were mycoplasma-free tested, were grown in RPMI 1640 medium supplemented with 10% heat-inactivated FBS. When the viability of the cells were found to be less than 20%, cell-free culture supernatants were harvested and filtered through a 0.45 mm pore size filter, and the viral particles were further purified by ultracentrifugation. The virus pellet was suspended in 5 mM sodium phosphate (pH 7.5) and purified by centrifugation on a 10 to 30% (wt/vol) dextran gradient. Concentrated viral preparations were resuspended in RPMI 1640, aliquoted and stored at −80° C. until used. Viral titers were found to be 1×10^7 transforming units (TFU)/ml. The results shown in FIGS. 2*a*, 2*b* and 2*c* are further explained below:

FIG. 2*a*: a: Groα(100 nM); b: EBV; c: IL-8 (100 nM)

FIG. 2*b*: a: Groα(100 nM); b: IL-8 (100 nM); c: EBV

FIG. 2*c*: a: EBV 1/10 dilution, 10^5 TFU/ml; b: EBV; c: Groα(100 nM); d: IL-8 (100 nM).

EXAMPLE 3

Effect of Pertussis Toxin on the Ca^{2+} Mobilization and De Novo RNA Synthesis Induced by EBV in Human Neutrophils

Human neutrophils (10×10^6 cells/ml) were preincubated 2 hours at 37° C. in the presence of 0.5 mg/ml of pertussis toxin (which catalyzes the ADP-ribosylation of G-protein and inhibits G-protein-mediated events) during two hours, prior to EBV (10^6 TFU/ml) or IL-8 (100 nM) stimulation. Calcium mobilization was measured as described in Example 2. Results are set out in FIG. 3*a* in which:

a: stimulation with IL-8;

b: stimulation with EBV;

c: pretreatment with pertussis toxin and stimulation with IL-8;

d: pretreatment with pertussis toxin and stimulation with EBV.

EBV-induced RNA synthesis was studied by measurement of the incorporation of [5-^{3}H] uridine into total RNA. Neutrophils (5×10^6 cells/ml) pretreated or not with pertussis toxin (0.5 mg/ml) were suspended in HBSS buffer supplemented with 1% heat-inactivated (1 h, 56° C.) autologous plasma. One hundred ml aliquotes of the cell suspensions were incubated in 96-well microtiter plates in the presence of 1 mCi of [5-^{3}H] uridine (per sample) and treated with 3 nM of GM-CSF (positive control) or with infectious EBV (10^6 TFU/ml). Plates were incubated during five hours at 37° C. under a humid atmosphere containing 5% CO_2. Following this incubation period, cells were harvested by filtration through glass fiber discs and radioactivity was measured in a liquid scintillation counter. Pertussis toxin was obtained from Sigma Chemicals (St. Louis, Mo.) and used in solution in NaCl 0.9% containing 0.01% BSA. Grocz was obtained from Peprotech and used as described above for pertussis toxin.

The results illustrated in FIG. 3*b* are representative of six different experiments (GM-CSF, granulocyte-macrophage colony-stimulating factor).

We claim:

1. A method for the treatment of a viral infection in a human or animal comprising administering to a human or animal in need of such treatment, a pharmacologically acceptable, therapeutically effective amount of an interleukin-8 agent, wherein said viral infection is of a DNA virus selected from the group consisting of parvoviridae, papovaviridae, adenoviridae, herpesviridae, poxviridae and hepadnaviridae; with the proviso that IL-8 agent excludes peptides of about 17 amino acids having the following formula:

$$\text{Glu-Leu-Arg-Cys-Xaa}_1\text{-Cys Xaa}_2\text{-Xaa}_3\text{-Xaa}_{4-Xaa5}\text{-Xaa}_6\text{-Xaa}_7\text{-}$$
$$\text{Xaa}_8\text{-Xaa}_9\text{-Xaa}_{10}\text{-Xaa}_{11}\text{-Xaa}_{12}$$

wherein:

Xaa_1 is Gln, Met, or Val;

Xaa_2 is Ile, or Val;

Xaa_3 is Lys, Gln, or Ser;

Xaa_4 is Thr, or Ile;

Xaa_5 is Tyr, Leu, Met, His, Val, or Thr;

Xaa_6 is Ser, Gln, Thr, or Ala;

Xaa_7 is Lys, Arg, or His;

Xaa_8 is absent or is Pro, Phe, or Gly;

Xaa_9 is absent or is Phe, Ile, or Val;

Xaa_{10} is absent or is His, Lys, or Arg;

Xaa_{11} is absent or is Pro, Leu, or Phe; and

Xaa_{12} is absent or is Lys, His or Arg.

2. A method according to claim **1** wherein said agent is interleukin-8 protein or an analog thereof which elicits an interleukin-8 biological response.

3. A method for the treatment of a viral infection in a human or animal comprising administering to a human or animal in need of such treatment, a pharmacologically acceptable, therapeutically effective amount of an interleukin-8 agent, wherein said viral infection is of an RNA virus selected from the group consisting of picornaviridae, togaviridae, orthomyxoviridae, paramyxoviridae, coronaviridae, reoviridae, oncornaviridae and filoviridae; with the proviso that IL-8 agent exclude peptides of about 17 amino acids having the following formula:

$$\text{Glu-Leu-Arg-Cys-Xaa}_1\text{-Cys Xaa}_2\text{-Xaa}_3\text{-Xaa}_4\text{-Xaa}_5\text{-Xaa}_6\text{-Xaa}_7\text{-}$$
$$\text{Xaa}_8\text{-Xaa}_9\text{-Xaa}_{10}\text{-Xaa}_{11}\text{-Xaa}_{12}$$

wherein:

Xaa_1 is Gln, Met, or Val;

Xaa_2 is Ile, or Val;

Xaa_3 is Lys, Gln, or Ser;

Xaa_4 is Thr, or Ile;

Xaa_5 is Tyr, Leu, Met, His, Val, or Thr;

Xaa_6 is Ser, Gln, Thr, or Ala;

Xaa_7 is Lys, Arg, or His;

Xaa_8 is absent or is Pro, Phe, or Gly;

Xaa_9 is absent or is Phe, Ile, or Val;

Xaa_{10} is absent or is His, Lys, or Arg;

Xaa_{11} is absent or is Pro, Leu, or Phe; and

Xaa_{12} is absent or is Lys, His or Arg.

4. A method according to claim **1** wherein said agent is interleukin-8 protein of 79 amino acids.

5. A method according to claim **1** wherein said agent is interleukin-8 protein of 72 amino acids.

6. A method according to claim **1** wherein said viral infection is of herpesviridae selected from the group consisting of EBV, HSV-1, HSV-2, CMV, VZV, HHV-6, HHV-7 and HHV-8.

7. A method according to claim **3**, wherein said viral infection is of togaviridae which is a bovine diarrhea virus.

8. A method according to claim **3**, wherein said viral infection is of picornaviridae which is a porcine enterovirus.

9. A method according to claim **3**, wherein said viral infection is of paramyxoviridae or bovine respiratory syncytial virus.

10. A method according to claim **1** wherein said agent is administered in conjunction with an antiviral agent selected from the group consisting of interferon -α, -β, -γ, tumor necrosis factor-α, ganciclovir, acyclovir, vidarabine, idoxuridine, prostaglandins and prostaglandin analogs.

11. A method according to claim **1** wherein said human or animal is an immunosuppreased patient or animal, or a patient treated with a drug known to enhance the occurrence of viral infections.

12. A method according to claim **11**, wherein said drug is selected from the group consisting of azathioprine, corticosteroids, adriamycine and methotrexate.

13. A method for the treatment of cancer caused by oncoviruses in a human or animal comprising administering to a human or animal in need of such treatment, a pharmacologically acceptable, therapeutically effective amount of interleukin-8 agent.

14. A method according to claim **13** wherein said agent is administered in combination with an anticancer agent selected from the group consisting of adriamycine, cyclophosphamide and methotrexate.

15. An antiviral pharmaceutical formulation comprising a pharmacologically acceptable, therapeutically effective amount of an interleukin-8 agent, in association with a pharmaceutically acceptable carrier.

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